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Short communication

Capillary electrophoresis of plant starches as the iodine complexes

Abstract

Much research is currently focused on the use of plant starch as an industrial raw material which can reduce dependence on non-renewable resources. Development of new starch-based products requires effective methods for analysis and characterization of starch and related biopolymers. Most separations methods are not suitable for this application due to the high molecular mass ($>10^6$) of starch. We describe here the use of iodine complexation to impart charge and permit detection of starch components in capillary electrophoresis. Amylopectin and amylose are resolved in less than 10 min using iodine-containing buffers in unmodified capillaries. Partial resolution of an oligosaccharide mixture was also demonstrated, indicating potential utility of the method for analysis of smaller biopolymers. The primary basis for separation is shown to be iodine binding affinity, which can be manipulated through control of temperature and iodine concentration.

1. Introduction

Plant starches and other polysaccharides comprise the most abundant source of renewable natural products on the planet [1]. Starch consists primarily of two components, amylose and amylopectin. Amylose is a linear or very lightly branched polymer consisting of several thousand (1→4)-α-D-linked D-glucose units with molecular masses ranging from $2 \cdot 10^5$ to $2 \cdot 10^6$ [2]. Amylopectin is a much larger, highly branched polymer consisting of relatively short (25 residue) segments of (1→4)-α D-linked-glucan units connected by (1→6)-α-D-glucosidic linkages, with molecular masses ranging from 10^7 to $5 \cdot 10^8$ [2,3]. Native plant starches typically contain 20–30% amylose, but the amylose content may range from 0 to 80% [2]. In potato and some other species a small fraction (<1%) of the glucose residues in amylopectin are phosphorylated [3].

The primary end-use of starch is in food products, with non-food uses accounting for approximately $2 \cdot 10^9$ kg of starch per year in the USA. Newer applications of starch in areas such as superabsorbent polymers [4] and biodegradable films [5] are emerging, but account for a small fraction of current starch production. There is great interest in expanding non-food uses of starch and other biopolymers, both to reduce dependence on non-renewable materials such as petroleum, and to provide new, high-value markets for agricultural products. Rapid and effective techniques for polysaccharide

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characterization are needed to support development of new starch applications, and to facilitate fundamental research on biopolymers. The tools currently used for characterization of polysaccharides include classical chemical techniques [6] and size-exclusion chromatography (SEC) [7]. The classical methods provide useful structural and chemical data, but can only determine average properties of the sample components. Chromatographic methods such as SEC can provide data on physical, chemical and structural properties of each component in a complex polysaccharide mixture [8]. Unfortunately, SEC separations provide limited resolution, and the high-resolution methods commonly used in carbohydrate analysis, such as reversed-phase HPLC [9], ion-exchange HPLC [10], and gel electrophoresis [11], are only useful for analytes with molecular masses much smaller than that of starch. High-molecular-mass polysaccharides have been separated using paper electrophoresis and related techniques [12], but this approach is less than ideal in terms of resolution, analysis time and detection capability.

Capillary electrophoresis (CE) has proven to be a very powerful tool for characterization of biomolecules [13]. Highly efficient separations of proteins [14] and nucleic acids [15] are now routinely obtained with CE. The application of CE to carbohydrate separations has not developed to the same degree, largely due to the lack of functional groups containing chromophores and charges. Simple sugars at relatively high concentrations have been separated as the anionic borate complexes [12,16,17] using CE with UV absorbance detection [18]. Oligosaccharide–borate complexes also have been separated, but derivatization with UV absorbing [19] or fluorescent [20,21] labels is required for detection. The derivatization procedures are lengthy (4–24 h), and detection of larger polysaccharides is problematic since only one label is typically introduced per molecule. Separations based on borate complexation appear to be limited to a maximum molecular mass of ca. 30 000 because borate complexes of larger molecules acquire a constant mass-to-charge ratio and are not resolved [22]. Only a few CE separations of larger polysaccharides have been reported. Borate complexes of synthetic mixtures of monodisperse dextrans have been resolved through use of pulsed-field conditions and sieving media with laser-induced fluorescent detection [22]. Fluorescent labeling was required, and the generality of the approach is not yet known. Unlabeled polysaccharides have been separated by CE under constant field conditions using indirect fluorescence detection at pH 11.5 [23]. In this case different classes of polysaccharides (e.g. amylose, pectin, dextrin) could be resolved, but each class migrated as a single peak.

An alternative strategy for imparting charge and optical detection sensitivity to carbohydrates is through iodine complexation. The starch–iodine complex consists of a helix of sugar residues surrounding a linear I_5^- core [24,25]. Unlike borate complexation, iodine binding is a cooperative interaction which exhibits strong chain-length dependence in both complexation and optical properties. The iodine binding constant increases nearly exponentially with glucan chain length, reaching a plateau at ca. 125 residues [26]. The wavelength of maximum absorbance (λ_{max}) also increases with chain length, varying from 496 nm for degree of polymerization (DP) 22 to 642 nm for DP 1500 [27]. The binding constant for amylose is several orders of magnitude higher than that of amylopectin, reflecting the much shorter average segment length in amylopectin. At sufficiently high iodine concentrations, however, both polymers bind approximately 20% of their mass in iodine at 20°C. Iodine binding and λ_{max} are also temperature dependent, especially for shorter chains. For example, the iodine binding capacity for a glucan of DP 31 increases almost tenfold on changing the temperature from 20.4 to 1.4°C [28]. Although iodine complexation was used over 40 years ago for agar gel electrophoresis of oligosaccharides [29] and free solution zone electrophoresis of cyclodextrins [30], this approach has not been exploited for the separation of high-molecular-mass polysaccharides. We describe here experiments which indicate that this strategy is applicable to the separation by CE of a wide range of carbohydrates ranging from

maltooligosaccharides of DP < 40 to amylopectins with molecular masses in the tens of millions. These separations can be achieved without derivatization or capillary modification, and with the speed, flexibility and small sample volumes typical of CE.

2. Experimental

2.1. Apparatus

CE was conducted with a Spectra Phoresis 1000 instrument (Thermo-Separations, San Jose, CA, USA) equipped with an autosampler, column temperature control, and UV–visible detector. The detector was generally operated in the multi-wavelength mode (simultaneous acquisition of data at several wavelengths), and occasionally in the high-speed scanning mode (data acquisition over a wide wavelength range at 5-nm increments) for acquisition of spectra. The fused-silica capillaries used were 50 μm I.D. × 340 μm O.D., with overall length of 36 cm and injector to detector length of 28 cm.

2.2. Reagents and solutions

All chemicals were used without further purification. Potato starch, amylose and amylopectin were from Sigma (St. Louis, MO, USA). Maltodextrin M-040, a mixture of maltodextrin oligomers with DP 1–40, was the gift of Dr. A. Hotchkiss, US Department of Agriculture, Philadelphia, PA, USA. Amaizo V (amylomaize V, apparent amylose content 50%) was obtained from American Maize Products (Chicago, IL, USA). Cornstarch was purchased at a local market. All other chemicals were reagent grade. Solutions were prepared with 18 MΩ water obtained from a Nanopure (Barnstead, Dubuque, IA, USA) water-purification system.

2.3. Procedures

Stock solutions of saccharides were prepared by magnetic stirring of a mixture containing 20 mg/ml of the analyte in dimethyl sulfoxide (DMSO) at room temperature until gelatinization (dissolution) was complete. Iodine stock solution contained 10 mg/ml KI and 10 mg/ml I_2. The stock solutions were stored at room temperature until needed. Shortly before analysis, an aliquot of the sample stock solution was diluted with water and buffer stock (50 m*M*) solution to give a sample of the appropriate composition. Run buffers were prepared by mixing buffer stock solution (50 m*M*), water, and iodine stock solution in the appropriate amounts shortly before use. The capillary was rinsed with run buffer using the instrument vacuum system for 2 min (ca. 3 column volumes) prior to each run. Injections were made by applying vacuum to the detector end of the capillary using the instrument injection system. The capillary was cleaned daily by rinsing for 10 min each with 1 *M* NaOH, water and run buffer.

3. Results and discussion

Typical electropherograms of amylose, amylopectin and potato starch are shown in Fig. 1. Amylopectin and amylose are well resolved from each other, with amylose migrating as a broad peak with electrophoretic mobility about 5 times higher than amylopectin. The electrophoretic mobility (adjusted for electroosmotic flow) of amylopectin was dependent on iodine concentration, as shown in Table 1. This dependence reflects the iodine binding behavior of amylopectin determined by titrimetry [28], and results from the short average chain length of amylopectin segments. Amylopectin mobility was also dependent on temperature (Table 2), commensurate with the strong temperature dependence of iodine binding of shorter segments. The mobility of amylose was virtually independent of iodine concentration and temperature (except for a viscosity-related mobility increase with temperature) over the same range, reflecting the fact that the long chains of amylose are essentially saturated with iodine even at very low iodine concentrations.

The broad distribution of migration times of the amylose fraction was unexpected. Since the

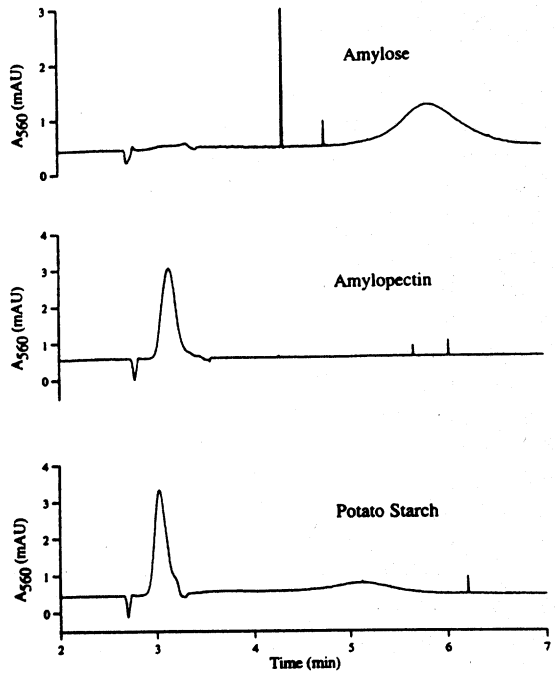

Fig. 1. Electropherograms of amylose, amylopectin and potato starch. Applied voltage: 20 kV; temperature: 25°C; injection: 5 s hydrodynamic; sample concentration: 1 mg/ml; run buffer: 20 m*M* citrate–phosphate, pH 6, 0.1 mg/ml KI, 0.1 mg/ml I_2.

iodine binding affinity of segments with DP > 100 is essentially constant, and the average segment length is ca. 200 for amylose, it was anticipated that all amylose molecules would acquire a constant charge-to-mass ratio and migrate at a uniform velocity. Peak broadening due to wall interactions can not be ruled out, but seems unlikely since both the capillary wall and the amylose–iodine complex carry a negative charge. Addition of hydroxypropyl cellulose (0.1%) to the run buffer, an agent known to reduce analyte–wall interactions, did not alter the amylose peak width, nor was peak width markedly effected by pH in the range 4–7.5 (data not shown). This evidence suggests that the distribution in mobility is due to sample heterogeneity, and not wall interactions, but further investigation is required to elucidate the mechanism for the dispersion in amylose mobility.

Table 1
Iodine concentration dependence of amylopectin and amylose mobility

$[I_2]$ (mg/ml)	Mobility (10^{-6} m^2 V^{-1} s^{-1})		Ratio
	Amylopectin	Amylose	
0.05	2.5	13.7	5.5
0.2	6.4	14.9	2.3
0.5	8.4	14.8	1.8
1.0	8.5	14.2	1.7

Conditions: applied voltage, 20 kV; temperature, 25°C; injection, 5 s hydrodynamic; sample concentration, 1 mg/ml; run buffer, 20 m*M* pH 5 acetate buffer containing indicated concentration of iodine. (Apparent mobilities corrected for electroosmotic flow).

Table 2
Temperature dependence of amylopectin mobility

Column temperature (°C)	Amylopectin mobility (10^{-6} m^2 V^{-1} s^{-1})
25	8.10
45	7.50
60	6.30

Conditions: applied voltage, 20 kV; injection, 5 s hydrodynamic; sample concentration, 1 mg/ml; run buffer, 20 m*M* pH 7 citrate–phosphate, 0.5 mg/ml I_2. (Apparent mobilities corrected for electroosmotic flow).

Spectra acquired during the runs (not shown) exhibited absorbance maxima of 560 nm for the amylopectin peak and 640 nm for the amylose peak. Spectra acquired at various points between the half maxima of both peaks appeared identical. Spectra at points near the base of the major peaks have absorbance maxima differing from the peak maximum, but the low signal-to-noise ratio of these spectra made accurate determination of the maximum value difficult. The dependence of absorbance maximum on chain length proved to be very useful in identifying components and can be an effective aid in developing and interpreting separations using iodine complexation. However, variations in λ_{max} and extinction coefficient with chain length must be born in mind when performing quantitative studies.

Separation of several starches and a mixture of

maltodextrin oligomers with a pH 7 citrate–phosphate buffer system are shown in Fig. 2. The partial separation of maltodextrin oligomers indicates the wide range of polymer molecular masses which can be studied by iodine complexation CE. The tall, narrow peaks migrating between amylopectin and amylose are observed in most amylose-containing samples, and to a lesser extent in amylopectin samples. The number and height of the peaks increase with sample age, while the exact number and location of the peaks varies from preparation to preparation and run to run. These peaks are believed to be associated with retrogradation (precipitation) of the amylose. Aqueous starch solutions are generally not stable, and tend to form crystalline solids or gels on standing. Relatively stable solutions of starch can be prepared in DMSO, and samples were prepared by dilution of the stock solutions in DMSO with aqueous electrolyte shortly before the separation to minimize retrogradation.

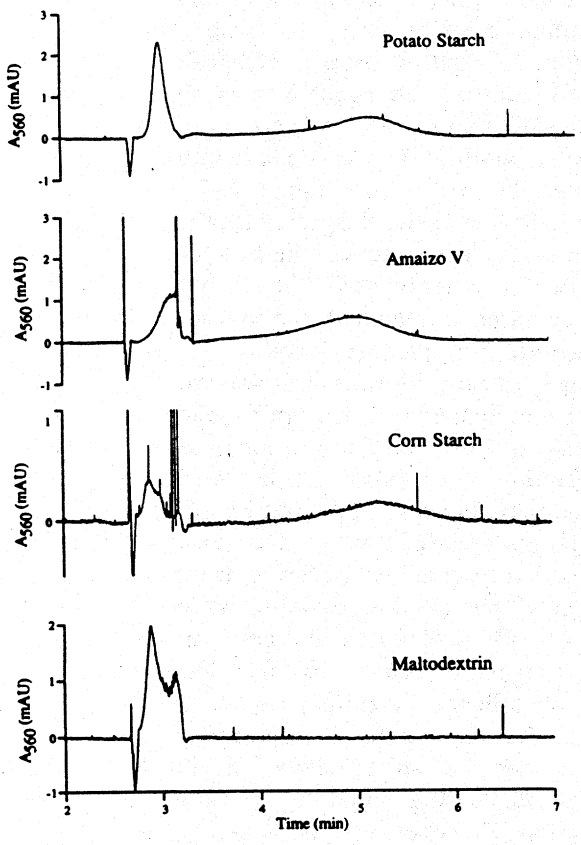

Fig. 2. Electropherograms of potato starch, Amaizo V, corn starch and maltodextrin M-040. Applied voltage: 20 kV; temperature: 25°C; injection: 5 s hydrodynamic; sample concentration: 1 mg/ml; run buffer: 20 m*M* citrate–phosphate, pH 7, 0.1 mg/ml KI, 0.1 mg/ml I_2.

Separations have been conducted in a number of buffers (acetate, phosphate and citrate–phosphate) over the pH range 4–7.5. Results similar to those shown in Fig. 1 were observed with all buffers, but differences in electroosmotic flow and small changes in peak shape were observed with various buffers. Higher-pH buffers appeared to lose iodine rapidly and gave irreproducible results. Iodine concentrations from 0.05 to 5 mg/ml have been used, with 0.1–0.5 mg/ml appearing optimal. At lower iodine concentrations amylopectin is not resolved from the void peak, while Joule heating due the high conductivity of KI solutions prevented separations in 50 μm capillaries at higher concentrations.

4. Conclusions

CE of starches using iodine-containing buffers provides a simple, rapid method for separation and detection of the principal starch components. In contrast to other approaches for CE of carbohydrates, lengthy (4–24 h) derivatization steps are avoided, and separations can be carried out in unmodified silica capillaries using commonly available detectors. The method can be used with relatively low-molecular-mass oligosaccharides as well as with high-molecular-mass polysaccharides which cannot be resolved using borate complexation [22]. The primary mechanism for selectivity is iodine binding affinity, and this can be manipulated by varying temperature and iodine concentration. Variations in iodine binding capacity and λ_{max} can reveal information on the size and structure of the analytes independent of their electrophoretic behavior. While much work is required to optimize separations and improve reproducibility, this approach appears very promising as a simple, rapid method for starch characterization.

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